Crystal Structure of the Lac Repressor Bound to Operator DNA at 2.6 Å Resolution

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Beamline(s): **X25**

Introduction: In previous work, crystal structures of the *E. coli* Lac repressor bound to operator and to the inducer isopropylthiogalactoside (IPTG) provided a model for how the binding of the inducer reduces the affinity of the repressor for operator. However, because of the low resolution of the operator-bound structure (4.8 Å), the model for the allosteric transition was presented in terms of structural elements rather than in terms of side chain interactions. The goal of this work was to extend the resolution of the operator-bound structure of the Lac repressor to 3.0 Å or better, in order to improve our understanding of the allosteric transition.

Methods and Materials: Residues 1-333 of the Lac repressor were expressed with an N-terminal 6-His tag and an intervening TEV proteolytic site using a T7 polymerase-driven expression vector. This dimeric version of the Lac repressor was purified by nickel chromatography, reacted with TEV protease, and concentrated to 14 mg ml^{-1}. A self-complementary 21 nucleotide *lac* operator with the sequence 5'-GAATTGTGAGCGCTCACAATT-3' was synthesized and HPLC-purified. The Lac repressor-operator complex was crystallized in the presence of the anti-inducer orthonitrophenylfucoside (ONPF). Crystals grew in space group R32 with cell dimensions a = 251.4Å, c = 204.8Å, with 1.5 dimeric repressor-DNA-ONPF complexes in the asymmetric unit and a solvent content of 75%. A native data set was collected to 2.6 Å at beamline X25, and the structure was determined by molecular replacement combined with solvent flattening and 3-fold averaging.

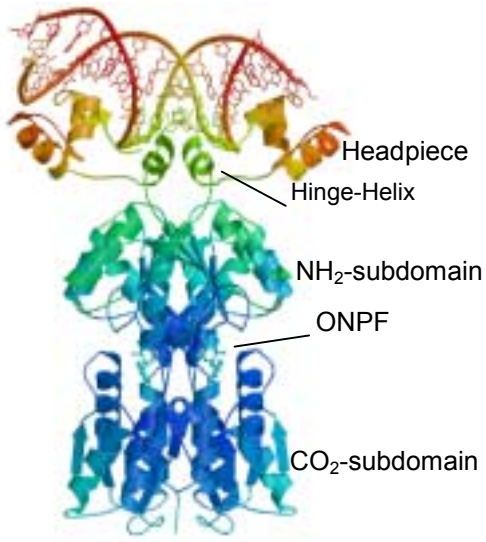

Figure 1. Structure of the dimeric Lac repressor complexed to operator. The structure is color coded according to temperature factor (red high and blue low). Binding of the inducer IPTG to the core domain (between the NH_2- and CO_2-subdomains) causes a conformational change that lowers the affinity of the repressor for operator. In this structure, the anti-inducer ONPF is bound to the inducer-binding pocket.

Results: In order to achieve the high-resolution structure of the Lac repressor-operator complex, two tricks were employed. First, a dimeric version of the repressor was constructed by deleting the C-terminal tetramerization helix. Second, the repressor-operator complex was crystallized in the presence of the anti-inducer ONPF, which binds to the same site on the repressor as IPTG, but increases (rather than decreases) the affinity of the repressor for operator. The 2.6 Å resolution data collected on frozen crystals at beamline X25 was a significant improvement over data collected at home (~3.5Å) or at other beamlines. The resulting electron density maps enabled placement of the side chains in the repressor operator complex. This allowed, for the first time, a detailed comparison of the conformation of the repressor in the repressed and induced states. In particular, the interactions at the dimer interface of the repressor, which are altered upon IPTG-binding, were visualized in detail. An extensive network of interactions between the DNA-binding and core domains of the repressor suggests a possible mechanism for the allosteric transition.

Conclusions: The 2.6 Å structure of the Lac repressor bound to operator and anti-inducer ONPF provides an improved framework for understanding the allosteric transition.

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References: C. Bell and M. Lewis, "A Closer View of the Conformation of the Lac Repressor Bound to Operator," Nature Structural Biology, 7, 209-214, 2000.